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VALUE OF FIELD DATA FOR EXTRAPOLATION IN ANTHRAX

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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland 21701

TECHNICAL MANUSCRIPT 349

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Process Development Division
AGENT DEVELOPMENT AND ENGINEERING LABORATORY

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February 1967

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

VALUE OF FIELD DATA FOR EXTRAPOLATION IN ANTHRAX

ABSTRACT

Data are presented to support the hypothesis that animals resistant to the establishment of anthrax are susceptible to its toxin, the former shown by dose of organisms and the latter by challenge with sterile toxin, and by the number of organisms and units of toxin per ml in terminal blood. The variables discussed are dose, doubling rate in the blood, terminal number of organisms per ml of blood, units of toxin per ml of terminal blood, inhibition of phagocytosis by toxin, spore germination within the phagocyte, quantitative phagocytosis in vitro, and lysis of phagocytes in vitro. The need for quantitative information from field cases of anthrax is emphasized for its usefulness as research information per se and to more completely understand field anthrax. In addition, the information obtainable by a field serological survey and its use are discussed.

One of the unfortunate generalizations that we may make of the literature on anthrax is that it is largely descriptive and it almost completely lacks quantitative information. Even in laboratory experiments, animals are reported as unobserved for long periods of time, which make questionable any statements regarding the time of death or specific response at death.

Experimental data leads to the hypothesis or model that species naturally fall into two classes: (i) those resistant to establishment of anthrax, but, once it is established, susceptible to the toxin; and (ii) the converse situation, species susceptible to the establishment of the disease but resistant to the toxin. The minimum data required for placing a species into the category of resistant or susceptible to the establishment of anthrax will be indicated. Information that may be obtained from a serological survey is also discussed.

Since data on the blood levels of bacilli and toxin at death are available, the relationship between these two variables can be presented as well as the more extrapolative aspects of this information. Table 1 shows that (i) each species has a characteristic rate of septicemic development, (ii) death occurs when the number of bacilli in the blood reaches a predetermined number, and (iii) the units of toxin are directly

related to number of organisms per ml of blood. The septicemic doubling rate does not change with changes in resistance attributed to immunity (guinea pig and rat); however, the number of organisms and units of toxin per ml of blood at death increase. Further information showing that the terminal number of organisms in the blood of guinea pig and rhesus monkey is directly related to the toxin level is given in Figures 1 and 2. This relationship can be influenced by time of death (Fig. 2) in that the shorter the time to death, the higher the number of organisms and units of toxin per ml of blood at death, and, conversely, the longer the time to death, the lower the number of organisms and units of toxin per ml of blood.[1] The dose-response relationship of the rat to sterile toxin (Fig. 3) also supports this generalization.

TABLE 1. QUANTITATIVE DYNAMICS OF THE SEPTICEMIC PHASE OF ANTHRAX

Species	Doubling Time		Terminal Blood Organisms per ml		Units of Toxin/ml
	Min.	Conf. Interval	No.	Conf. Interval	
Mouse	45	37 - 59	$1 \times 10^{7.0}$	$10^{6.6}$ to $10^{7.4}$	-
Guinea Pig	53	41 - 73	$1 \times 10^{8.3}$	$10^{8.0}$ to $10^{8.6}$	80
Guinea Pig (Immune PA5)	53	-	5×10^{7}	-	55
Guinea Pig (Immune PA5 + LV)	53	-	$1 \times 10^{8.1}$	-	25
Rhesus Monkey	48	26 - 300	$1 \times 10^{8.8}$	$10^{8.5}$ to $10^{9.2}$	35
Chimpanzee	155	-	$1 \times 10^{8.9}$	-	110
Rat, NIH Black	120	102 - 139	$1 \times 10^{8.8}$	$10^{8.8}$ to $10^{8.2}$	-
Rat, Fischer 344	120	-	$1 \times 10^{9.8}$	$10^{9.6}$ to $10^{9.0}$	15
Rat, F344 (Immune PA5)	-	-	-	-	13
Rat, F344 (Immune PA5 + LV)	-	-	-	-	9

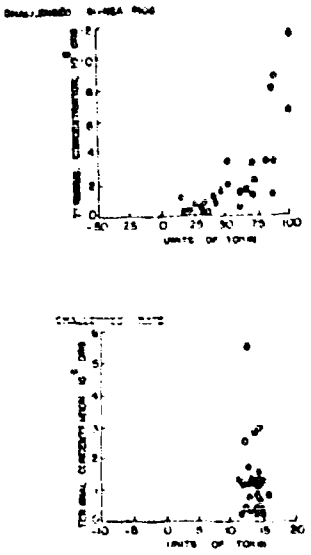

Figure 1. Interaction Among Terminal Variables in the Immunized Guinea Pig and Rat. Each data point represents one immunization protocol and is the mean of 9 or 10 animals distributed among two populations.

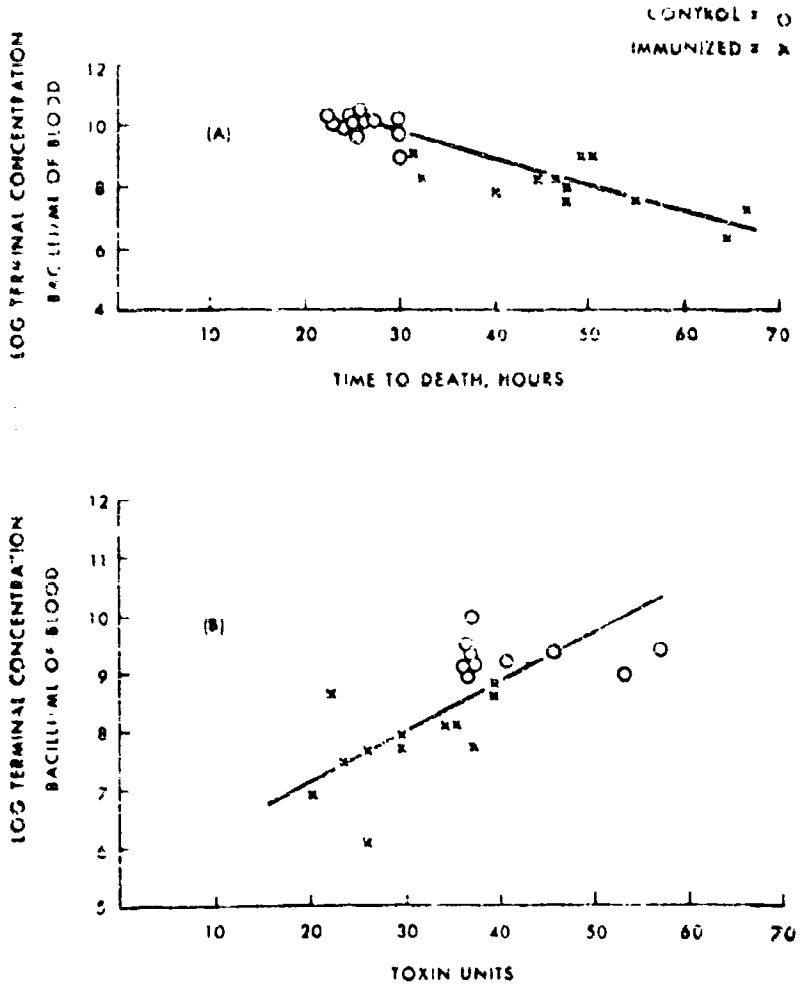

Figure 2. Relationship of Terminal Concentration of Organisms in Blood with Time to Death and Terminal Concentration of Toxin in Blood of Control and Immunized Guinea Pigs.

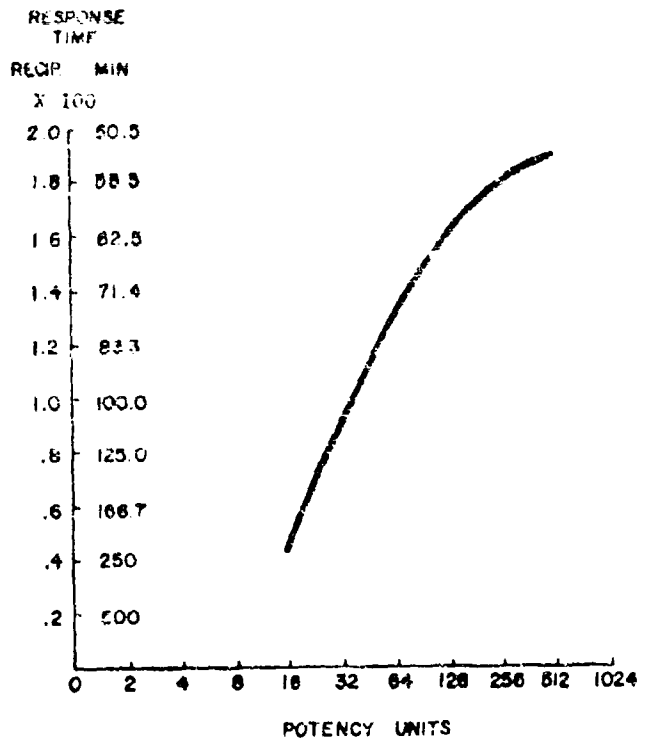

Figure 3. Regression of Reciprocal Response Time of Fischer Rats on Log Dose of Anthrax Toxins Expressed in Potency Units.

Certain generalizations may be made from these data. We know that once a septicemia is observed progression of the disease is rapid and predictable. Figure 4 is modified after Keppie, Smith and Harris-Smith,[2] and we have published similar data on several species. With the guinea pig, once the septicemia is detectable by observation of organisms on a blood smear, there is an average of 12 hours until death and about 4 hours in which treatment with streptomycin can be initiated with any expectation of recovery. Whether the host recovers or not depends upon the amount of toxin fixed. Keppie et al.[2] showed that after a critical level of about 1×10^5 to 3×10^5 organisms per ml was reached, treatment with streptomycin (which reduced the level of organisms in the blood to essentially the zero level) merely extended the time to death. Both rats[3] and monkeys[4] challenged with sterile toxin survived if antiserum was administered during the first third of the period between challenge and death (established by untreated control animals). If antiserum was given after this period, death still occurred; however, the time to death was extended. It is appropriate to

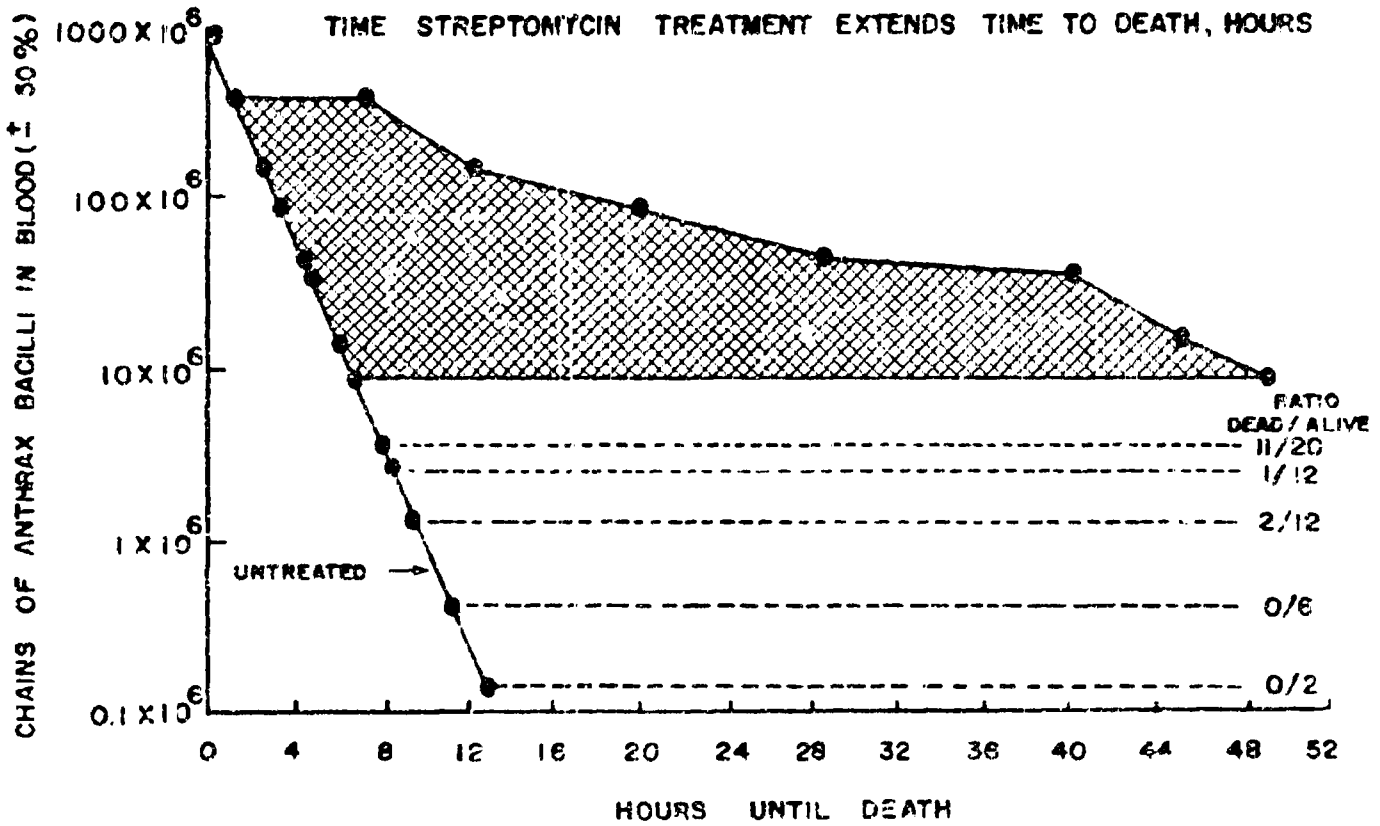

Figure 4. Relationship Between Level of Anthrax Septicemia and Time to Death (Guinea Pig). Change in death time by streptomycin treatment. (Data from Keppie et al.[4])

TABLE 2. RELATIONSHIP BETWEEN SUSCEPTIBILITY TO TOXIN CHALLENGE AND RESISTANCE TO ESTABLISHMENT OF ANTHRAX

Species	Units of Toxin/Kg Causing Death	Time to Death, hours	Relative Resistance to Parenteral Challenge of Spores[a]
Mouse	1000	24	very susc.
Guinea Pig	1125	24	susc.
Rabbit	2500	72	susc.
Rhesus Monkey	2500	28	susc.
Chimpanzee	4000	60	susc.
Rat, NIH Black	280	20	resistant
Rat, Fischer 344	15	2	resistant
Beagle	60	20	very resistant

a. Specific information given in Tables 3 and 6.

If one accepts the above basis for generalization, then the dose to establish anthrax infection is inversely related to the number of organisms per ml of blood at death. The data in Table 3 show that species that require a large dose to establish the disease have a low number of organisms in the blood at death, and vice versa. Although we believe that the units of toxin are of more significance than the number of bacilli, these data are still more difficult to collect. Nevertheless, we have shown a strong positive correlation between these variables. By obtaining quantitative information on the number of bacilli per ml of blood at death of any species of interest, a calculated prediction can be made as to the probable dose required to infect that host. Criticism could arise from the fact that we do not know the route of infection in field cases; however, it is our experience with numerous laboratory species that the route of infection does not in any way affect the terminal level of organisms. In any case, we suggest that a calculated realistic dose is better than continued ignorance; therefore, until data become available this relationship is a reasonable working model. It is, moreover, a working model readily susceptible to critical experimental examination.

TABLE 3. INVERSE RELATIONSHIP BETWEEN DOSE TO ESTABLISH ANTHRAX AND NUMBER OF ORGANISMS PER ML OF BLOOD AT DEATH

Species	Relative Resistance	Dose to Establish Parenteral Anthrax, spores	Quantitation of Blood at Death	
			Bacilli per ml	Toxin, units per ml
Mouse	Very susc.	5	$10^{8.9}$	-
Guinea pig	Susceptible	50	$10^{8.3}$	50
Rabbit	Susceptible	5000	$10^{8.0}$	-
Rhesus Monkey	Susceptible	3000	$10^{8.8}$	35
Chimpanzee	Susceptible	-	$10^{8.9}$	110
Rat	Resistant	$1x10^{8}$	$10^{4.8}$	15
Dog	Very resistant	ca. $50x10^{8}$	-	-

Certainly the proposal presented above is not the only approach to this problem. The ideal model would not result in the death of the host and, since blood can be readily obtained, it would be a very desirable system on which to construct an extrapolative model. The only known other attempt to assemble facts on anthrax so that some extrapolative evaluation may be made is the preliminary work by Rosenwald et al.* on changes in phagocytic and anthracidal activity of blood cellular components as influenced by anthrax toxin concentration. They attempted to extend the observations of Kashiba et al.[9] on the highest dilution of terminal guinea pig serum that gave positive inhibition of phagocytes from different species (Table 4). Those species having leukocytes most sensitive to toxin are several known to be susceptible to establishment of anthrax, the guinea pig, rabbit, mouse and sheep, with those unknown, man, cow and horse, included in this group; leukocytes of the more resistant species are very resistant to inhibition by toxin. Because Rosenwald et al.* could not duplicate the Japanese observations, they went on to survey other interactions between the phagocyte and spore. Table 5 presents two responses that were studied. The differences among species are interesting, but until combined with other data (Table 6) they have little consistency. When combined with the other quantitative information available on anthrax, there is definitely a difference between the species listed as susceptible or resistant. In the susceptible group, as regards dose required to establish anthrax, the terminal number of organisms is higher, inhibition of phagocytes by toxin is greater, a higher unit of toxin is required to kill by intravenous injection, and intracellular germination of spores

* Rosenwald, A.J.; Jones, W.I., Jr.; Lincoln, R.E. Unpublished data.

in phagocytes is consistently different from that in the resistant group. This is not a coincidence but an indication of general interrelationship of host-parasite interaction. The whole problem of extrapolation of disease response from experimental animals to man is so complex and difficult that we think it inappropriate to do more than suggest that the apparent relationship is real and that more work needs to be done to explore this model.

TABLE 4. SUSCEPTIBILITY OF LEUKOCYTES OF SEVERAL SPECIES TO ANTHRAX AGGRESSION

Species	Maximum Final Dilution of Positive Inhibition
Guinea Pig, Cow, Man, Rabbit, Sheep, Horse	1:32
Mouse	1:16
Rat	1:4
Dog, Swine	No Inhibition

Of all the contagious diseases serologically surveyed in the field, we know of no survey considering anthrax. This may be because of the few scientific workers interested in anthrax; it is also debatable that a good serology system has been developed. Perhaps it is tacitly assumed that all anthrax infections are lethal, an assumption that does not seem reasonable considering the uncertainty of biological responses and the prevalence of marginally virulent field strains and of resistant species. In addition, more than one cell of B. anthracis is required to cause infection, and cases of recovery have been reported. In South Africa, Sterne[10] reports that only 25% of anthrax deaths had been diagnosed and reported as indicated by results of blood smears taken of every animal that died in the area surveyed. Dordevic,[11] reporting on anthrax in man and animals in Yugoslavia, states that official data do not cover all the cases of anthrax and that the number of cases is easily double that reported. It is also recognized that for political and economic reasons a country may not report anthrax, although it may occur at a significant rate.

TABLE 5. SPORES GERMINATING INTRACELLULARLY, PHAGOCYTES CONTAINING >20 ORGANISMS AND PHAGOCYTES DESTROYED

Species	Germination Within Phagocyte, %	60 Minutes	
		Phagocytes With >20 Cells, %	Phagocytes Destroyed, %
Guinea Pig	35	34	19
Guinea Pig (Immune PA5)	-	29	32
Rhesus Monkey	25	0	4
Chimpanzee	25	12	50
Man	6	84	65
Man (Immune PA5)	0	3	15
Rat, NIH Black	24	8	0
Rat, Fischer 344	17	28	70
Cow	10	-	-
Horse	41	-	-
Sheep	49	-	-
Goat	68	-	-
Dog	9	-	-
Swine	33	-	-

TABLE 6. SUMMARY OF DATA RELATING SUSCEPTIBILITY IN ESTABLISHMENT OF ANTHRAX TO OTHER CRITICAL OBSERVATIONS

	Terminal Blood		Susc. Toxin Inhibition, dilution	Susc. to Toxin Challenge	Phagocytes			Approx. LD_{50} Dose[a]	
	Org/ml	Toxin, Units/ml			Germination Intracellular, %	Containing 20 Org, %	Destroyed, %	Parenteral	Aerosol
Susceptible Sp.									
Chimpanzee	$10^{8.9}$	110	1:32	V. Res.	25	12	50	-	50,000[b]
Guinea Pig	$10^{8.3}$	50	1:32	Res.	35	34	19	50	16,650
Rabbit	$10^{8.3}$	-	1:32	Res.	-	-	-	5000	35,000[c]
Mouse	$10^{7.3}$	-	1:16	Res.	-	-	-	5	14,500
Rhesus Monkey	$10^{8.11}$	35	-	Res.	25	0	4	3000	80,000
Goat	High[e]	-	1:32	-	68	-	-	"	-
Sheep	High	-	1:32	-	49	-	-	-	200,000[d]
Horse	High[e]	-	1:32	-	41	-	-	-	-
Cow	High[e]	-	1:32	-	10	-	-	-	-
Man	High[e]	-	1:32	-	6	84	65	-	-
Resistant Sp.									
Immune G. Pig	$10^{8.6}$	-	-	-	-	39	32	$1x10^{9}$	-
Rat, NIH Black	$10^{8.9}$	-	1:4	Susc.	24	~8	0	$1.5x10^{8}$ f/	-
Rat, Fischer 344	$10^{4.0}$	<8	1:4	V. Susc.	17	28	20	$0.7x10^{8}$ f/	255,000
Dog	-	-	1:0	Susc.	9	-	-	Ca. $5x10^{10}$ R/	$1.8x10^{7}$
Swine	-	-	1:0	-	33	-	-	$1x10^{9}$	$2.6x10^{7}$ h/
Immunized Man	-	-	-	-	0	3	15	-	-

a. Except as noted, from Young, Zelle, and Lincoln (1946).[7]
b. Albrink and Goodlow (1959).[8]
c. Est. from Young et al.[7] (1946) that rabbits and hamsters are two times as susceptible as guinea pigs.
d. Trnka et al. (1948).[9]
e. Estimate based on frequent mention of "large" number of organisms in blood taken at death.
f. Unpublished data.
g. Est. from Young et al.[7] (1946) four dogs given $1x10^{8}$ showed febrile reaction; one challenged at $1x10^{9}$ died.
h. Est. from Young et al.[7] (1946) resistance comparable to rat and dog.

A field survey would give much valuable information on incipient or controlled infections versus the observed or diagnosed infections. By obtaining both qualitative and quantitative information on antigens, such a survey would characterize B. anthracis to a far greater degree than has yet been done. Certainly, such characterization would establish (i) if a strain specialization for bovines, goats, etc., does exist and (ii) the prevalence of strains able to overcome the protective antigen type of immunization.[12]

The translation of disease models from experimental hosts to man or his domesticated animals might well be considered one of the most challenging and difficult problems for medical researchers. With a "lethal" disease, such as anthrax, the problems are greatly increased over those of a non-lethal one. Our comments have been made not specifically to urge or deny the value of a field test or survey, but rather to note that a relationship exists among experimental species that affects our view on the epidemiology and treatment of anthrax. Where quantitative data are available, there is reasonable support of this hypothesis; however, too little is known about man and the domesticated animals for the suggested model to be evaluated broadly. We hoped to show the type of quantitative and qualitative data needed to more completely evaluate field anthrax and thereby to accumulate such information so that an evaluation could reasonably be made of how the model discussed here applied to wild species endemically exposed to anthrax or to man or his domesticated species.

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13 ABSTRACT

Data are presented to support the hypothesis that animals resistant to the establishment of anthrax are susceptible to its toxin, the former shown by dose of organisms and the latter by challenge with sterile toxin, and by the number of organisms and units of toxin per ml in terminal blood. The variables discussed are dose, doubling rate in the blood, terminal number of organisms per ml of blood, units of toxin per ml of terminal blood, inhibition of phagocytosis by toxin, spore germination within the phagocyte, quantitative phagocytosis in vitro, and lysis of phagocytes in vitro. The need for quantitative information from field cases of anthrax is emphasized for its usefulness as research information per se and to more completely understand field anthrax. In addition, the information obtainable by a field serological survey and its use are discussed.

14. Key Words

*Anthrax	Septicemia	Rhesus monkeys
*Immunity	Experimental animals	Chimpanzees
*Toxins	Streptomycin	Humans
*Organisms	Infectivity	Rats
Dose	Leucocytes	Lethal
Serology	Spores	Mice
Phagocytes	Inhalation	Field tests
Blood	Guinea pigs	

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